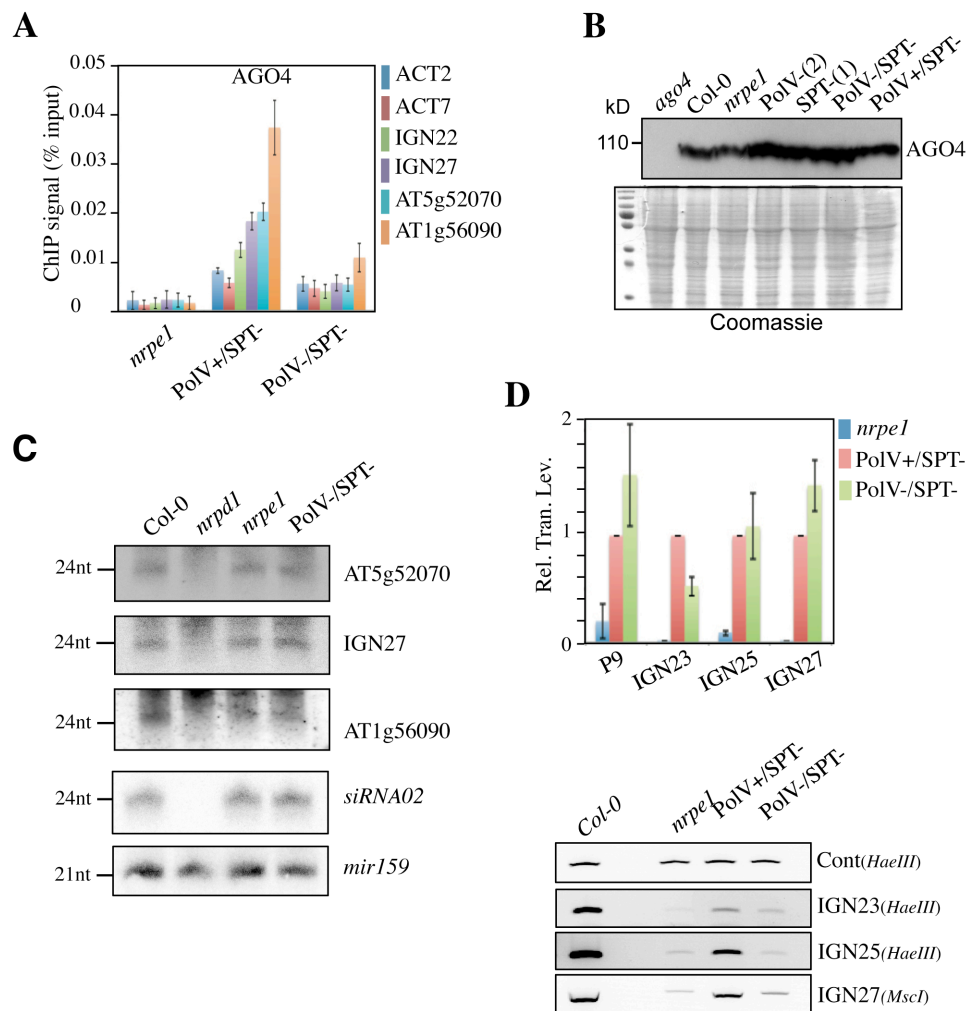


Supplemental_Fig_S4



Supplemental Fig. S4: AGO hooks and not P5RNAs are the major determinants of AGO4 recruitment to RdDM loci

A) Chromatin immunoprecipitation analysis of AGO4 binding using anti-AGO4 antibodies in *nrpe1*, PolV+/SPT- and PolV-/SPT- lines. The tested targets are indicated on the right. Actin2 and Actin7 are used as negative controls. Values are means +/-SD from two independent amplifications. B) Analysis of the AGO4 protein accumulation by western blots in *ago4*, Col-0, *nrpe1*, the PolV-(2) and SPT-(1) complemented lines and in PolV-/SPT- and PolV+/SPT- cross lines. Coomassie blue staining is used as a loading control. C) Analysis of siRNA levels by Northern blot in Col-0, *nrpd1*, *nrpe1*, and PolV-/SPT- lines. Mir159 is used as a loading control. D) *Top panel* P9, IGN23, IGN23, IGN27 transcript accumulation was tested in *nrpe1*, PolV+/SPT- and PolV-/SPT- lines. Rel. Tran. Lev. stands for Relative Transcript Level normalized to Actin and PolV+/SPT- using the $\Delta\Delta C_t$ method. *Bottom panel:* Analysis of DNA methylation by Chop-PCR at IGN23, IGN25 and IGN27 loci. Genomic DNA was digested with *HaeIII* or *MscI* methylation sensitive enzymes and used as template for PCR. The *RDRP* gene has no *HaeIII* site and was used as control (cont). DNA methylation was assessed in PolV+/SPT- and PolV-/SPT- cross lines in the right panel. Col-0 and *nrpe1* mutant were used as controls.